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Long-term efficacy of interferon alpha therapy on hepatitis B viral replication in patients with chronic hepatitis B: A meta-analysis

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ABSTRACT

Background/aims: Interferon (IFN) alpha has been used in the treatment of chronic hepatitis B for decades. Beneficial effects including hepatitis B e antigen (HBeAg)/HBV DNA seroclearance have been documented. However, it remains unclear whether interferon has long-term efficacy on inhibiting hepatitis B viral replication. So we conducted a meta-analysis of available literature to assess the evidence obtained on the efficacy of IFN treatment in chronic HBV infection.

Methods: Seven clinical controlled trials, including 1550 patients and comparing IFN to no treatment, were selected. Data on the incidence of HBV DNA seroclearance, HBeAg seroclearance, and HBsAg seroclearance in IFN treated and untreated patients were extracted from each study. The evaluation of effectiveness was performed with an intention-to-treat (ITT) method. We used the relative risk (RR) and 95% confidence interval (CI) of the main outcomes as the measure of efficacy. Meta-analysis was performed using fixed-effect or random-effect methods, depending on absence or presence of significant heterogeneity. Analyses were performed with STATA version 9.0 and Review Manager Version 4.2.

Results: Four studies including the data of HBeAg seroclearance with significant heterogeneity were analyzed by random-effect method; six studies including the data of HBsAg seroclearance without significant heterogeneity were analyzed by fixed-effect method. A different incidence of HBeAg seroclearance and HBsAg seroclearance was observed between treated and untreated patients. The RR of HBeAg seroclearance and HBsAg seroclearance was 0.66 (95% CI: 0.44, 0.99) and 0.28 (95% CI: 0.17, 0.46), respectively.

Conclusions: In conclusion, the results of this meta-analysis indicate that IFN increases the incidence of HBeAg seroclearance and HBsAg seroclearance after long-term follow-up of three to seven years.

1. Introduction

Hepatitis B virus (HBV) infection is a global public health problem as it is the leading cause of cirrhosis and hepatocellular carcinoma (HCC) with up to one million HBV carriers dying of HBV associated liver disease annually (Safioleas et al., 2007). Interferon (IFN) has been used in the treatment of chronic hepatitis B for decades. Beneficial effects after a 4–6 months of IFN treatment, including hepatitis B e antigen (HBeAg)/HBV DNA seroclearance, have been documented (Manns, 2002). Early controlled studies have shown that a 4- to 6-month course of conventional IFN-alpha at a dose of 5 million unit (MU) daily or 10 MU three times weekly achieved HBeAg loss in approximately 33% of HBeAg positive patients in comparison with 12% of controls (Wong et al., 1993).

The most recent meta-analyzed including 24 randomized controlled trials and concluded that the risk differences were in favor of interferon for persistent ALT normalization, clearance of HBeAg, and sustained loss of HBV DNA (by hybridization techniques) approximately 25% greater than for controls. After termination of IFN treatment, 20% of HBeAg positive patients and 70% of HBeAg negative patients will relapse in one year (Craxi et al., 2003). But it remains unclear how many patients will relapse beyond one year, and whether interferon has long-term efficacy on inhibiting hepatitis B viral replication in patients with chronic hepatitis B. Thus, we conducted this meta-analysis of available trials to assess the evidence obtained on the efficacy of IFN treatment in chronic HBV infection.

2. Methods

2.1. Literature search and data extraction

All articles were retrieved by using searches of PUBMED and EMBASE. Included terms were "interferon AND (hepatitis B OR HBV)". Our search was limited to human studies. All articles were

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identified by a search from 1986 to May 2009. Reference lists from all the available review articles, primary studies and proceedings of major meetings were also screened, in order to identify studies not found by the PUBMED and EMBASE search. All studies were initially reviewed using a list of predefined, pertinent issues concerning patients, features and treatments. Data were extracted from each study by two separate investigators (Y.F. Yang and Y.D. Zhong). Discrepancies among reviewers were infrequent (overall interobserver variations, 11%), and were solved with discussion. Basic information obtained from each eligible trial included the numbers of patients in each compared group at the outset of the trial, the characteristics of each group at base line (include female/male ratio, age, HBeAg status, serum alanine transarninase, preexist liver cirrhosis, etc.), the treatment regimes, duration of follow-up and the treatment outcomes at the end follow-up period. Articles were examined to eliminate duplicate reports of the same trial, and uncertainties in the data were clarified by contacting the principal investigators through writing when necessary.

2.2. Inclusion and exclusion criteria

Studies were included in the meta-analysis if:

(1) When reporting randomized controlled trials (RCTs) or non-randomized controlled trials (NRCTs), accepted for publication as full length papers or meeting's abstracts, following up IFN treated and untreated patients with chronic hepatitis B for more than three years.
(2) When providing sufficient analytical information of treatment schedule, follow-up and outcomes.
(3) When assessing HBV DNA seroclearance, HBeAg seroclearance and HBsAg seroclearance as outcome measures of the treatment effect.

Trials including patients suffering from other forms of viral hepatitis (hepatitis C or hepatitis D) or receiving antiviral drugs other than IFN were excluded.

2.3. Definition of main outcomes

HBV DNA seroclearance was defined as undetectable of HBV DNA in the serum. HBeAg seroclearance was defined as disappearance of HBeAg in the serum. Hepatitis B s antigen (HBsAg) seroclearance was defined as disappearance of HBsAg in the serum.

2.4. Statistical analysis

The evaluation of preventive effectiveness was performed with an intention-to-treat method (ITT, i.e. all patients were evaluated according to their allocated treatment group; patients whose endpoint was unknown were considered failures). We used the relative risk (RR) of the main outcomes as the measure of efficacy. The 95% confidence interval (CI) for the combined RR is also provided. Meta-analysis was performed using fixed-effect or random-effect methods, depending on absence or presence of significant heterogeneity (DerSimonian and Laird, 1986). Statistical heterogeneity between trials was evaluated by the Cochran χ^2 test and was considered to exist when $P < 0.10$. In the absence of statistically significant heterogeneity, the fixed-effect method was used to combine the results. When the heterogeneity test was statistically significant ($P = 0.10$ or lower), the random-effect method was used. The combined result was an average RR and 95% CI weighted according to the standard error of the RR of the trial, $P < 0.05$ was considered statistically significant. We used funnel plots (i.e. plots of study results against precision) to assess publication bias, and tested the symmetry of the funnel plot using Egger's test (Egger et

Table 1
Features of the included studies.

Study	Region	Study type	Include patients	Interferon regimens	Sample size (n)	Age (years)	Males/females	ALT (IU/L)	Preexist cirrhosis	Follow-up period
Lin et al. (2007)	Taiwan	Case control	eAg (+) CHB and Child A cirrhosis	6–9 MU for 11–16 w	T 233 C 233	T 32 ± 7 C 31 ± 8	T 219/14 C 219/14	T 175 ± 112 C 187 ± 109	T19/233 C 15/233	T 6.8 ± 3.2 y C 6.1 ± 3.0 y
Truong et al. (2005)	Japan	Cohort	eAg (+) and eAg (−) CHB	24 w	T 27 C 35	T 33.2 ± 10.4 C 36.6 ± 10.9	T 19/8 C 14/21	T 238.6 ± 250.1 C 142.3 ± 152.1*	No	T 7.0 ± 2.5 y C 6.2 ± 2.9 y
Papatheodoridis et al. (2001)	Greece	Cohort	eAg (−) CHB	3 MU for 6–12 m	T 209 C 195	T 46.8 ± 11.3 C 48.8 ± 13.7	T 174/35 C 160/35	T 112 (13–1905) C 68 (20–1335)*	T 57/209 C 68/195	T 5.6 ± 2.7 y C 6.0 ± 27 y
Mazzella et al. (1999)	Italy	RCT	eAg (+) CHB	5 MU/m 2 for 6 m	T 33 C 31	T 36.3 (18–64) C 40.6 (18–65)	T 25/8 C 25/6	T 106 ± 51 C 144 ± 90	No	T86.4 ± 6.96 m C 79.7 ± 6.8 m
Krogsgaard (1998)	Europe	Cohort	eAg (+) CHB	2.5–10 MU for 12–24 w	T 210 C 98	36 (16–65)	Female 19%	Elevated	19%	4.7 (0.2–7.5) y
Fattovich et al. (1997)	Europe	Cohort	eAg (+) cirrhosis	For 12–52 w	T 40 C 50	T 47 ± 1.8 C 45 ± 2.2	T 34/10 C 44/6	T 5.3 ± 0.6 ULN C 5.3 ± 0.6 ULN	100%	T 86 ± 5.99 m C 86 ± 6.71 m
Niederau et al. (1996)	Germany	Cohort	eAg (+) CHB	2–5 MU for 4–6 m	T 103 C 53	T 39.9 ± 1.9 C 42.8 ± 19	T 80/23 C 42/11	T 112 ± 13 C 108 ± 12	T 27/103 C 16/53	T 50.9 ± 19.8 m C 38.5 ± 18.2 m

Note: RCT, randomized controlled trial; CHB, chronic hepatitis B; eAg, e antigen; MU, million unit; T, treated group; C, control group; m, months; w, weeks; y, years
* Statistically significant.

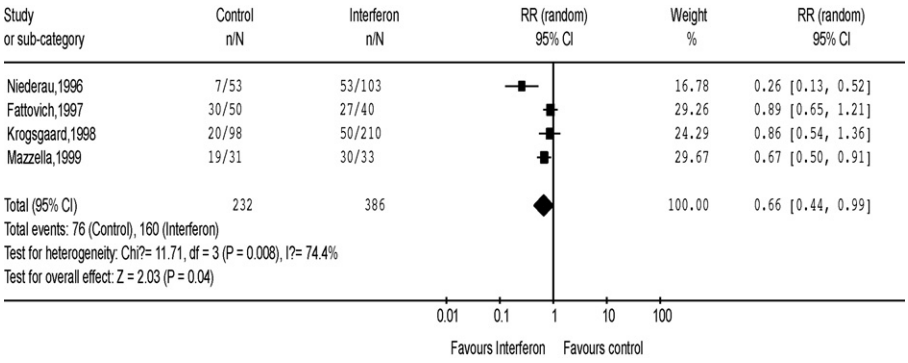

Fig. 1. Meta-analysis of the four trials comparing incidence of HBeAg seroclearance in untreated and IFN treated patients with chronic hepatitis B. Risk Ratio and 95% confidence intervals for each study and the pooled estimate of the treatment effect with its confidence interval are plotted on the graph. Studies are arranged by publication year.

al., 1997; Sterne and Egger, 2001). Analyses were performed with STATA version 9.0 (Stata Corp., College Station, TX) and Review Manager version 4.2 (RevMan, The Cochrane Collaboration, Oxford, England).

3. Results

3.1. Features of the studies

Nine potentially eligible RCTs and NRCTs following up IFN treated and untreated patients with CHB for more than three years were identified (Papatheodoridis et al., 2001; Mazzella et al., 1999; Krogsgaard, 1998; Fattovich et al., 1997; Niederau et al., 1996; Lin et al., 2004, 2007; Yuen et al., 2001; Truong et al., 2005). One smaller study (Lin et al., 2004) that published earlier was included in another study (Lin et al., 2007) published more recently, so the earlier study was excluded. One study enrolled asymptomatic HBsAg carriers (Yuen et al., 2001) was also excluded.

The main features of the studies evaluated by meta-analysis are shown in Table 1. Seven studies (Papatheodoridis et al., 2001; Mazzella et al., 1999; Krogsgaard, 1998; Fattovich et al., 1997; Niederau et al., 1996; Lin et al., 2007; Truong et al., 2005) fulfilled the requirements and included 1550 patients, 855 of whom received IFN treatment and 695 of whom received no treatment. Among the reviewed studies, one was RCT (Mazzella et al., 1999) and six were NRCTs (Papatheodoridis et al., 2001; Krogsgaard, 1998; Fattovich et al., 1997; Niederau et al., 1996; Lin et al., 2007; Truong et al., 2005). Patients' selection criteria were different among studies. Two studies (Mazzella et al., 1999; Truong et al., 2005) included only patients with HBeAg positive/negative chronic hepatitis B, four studies (Papatheodoridis et al., 2001; Krogsgaard, 1998; Niederau et al., 1996; Lin et al., 2007) included patients with HBeAg positive/negative chronic hepatitis B or Child-Pugh A cirrhosis, while one study (Fattovich et al., 1997) included only patients with HBV-related cirrhosis. The sample size of each study varied greatly, ranging from 62 (Truong et al., 2005) to 466 (Lin et al., 2007) patients. The mean age ranged from 32 (Lin et al., 2007) to 47 (Fattovich et al., 1997) years old for treated patients and from 31 (Lin et al., 2007) to 48.8 (Papatheodoridis et al., 2001) years old for untreated patients. The percentage of females ranged from 6.0% (Lin et al., 2007) to 46.8% (Truong et al., 2005). The length of follow up differed between studies, ranging from 38.5 months (Niederau et al., 1996) to seven years (Mazzella et al., 1999; Fattovich et al., 1997; Truong et al., 2005). Generally, age, male/female ratio, percentage of preexist cirrhosis, and length of follow up were not significant different between IFN groups and untreated groups in these studies. But the ALT levels were significantly higher in IFN groups than in untreated groups in two studies (Papatheodoridis et al., 2001; Truong et al., 2005).

There was no evidence for publication bias on the funnel plot (data not shown) or by Egger's test ($P > 0.1$).

3.2. The effect of IFN on HBV DNA seroclearance

For only three studies (Mazzella et al., 1999; Niederau et al., 1996; Truong et al., 2005) containing the information about incidence of HBV DNA seroclearance, meta-analysis has not been conducted. Generally, two (Mazzella et al., 1999; Niederau et al., 1996) of the three studies showed that IFN treatment significantly

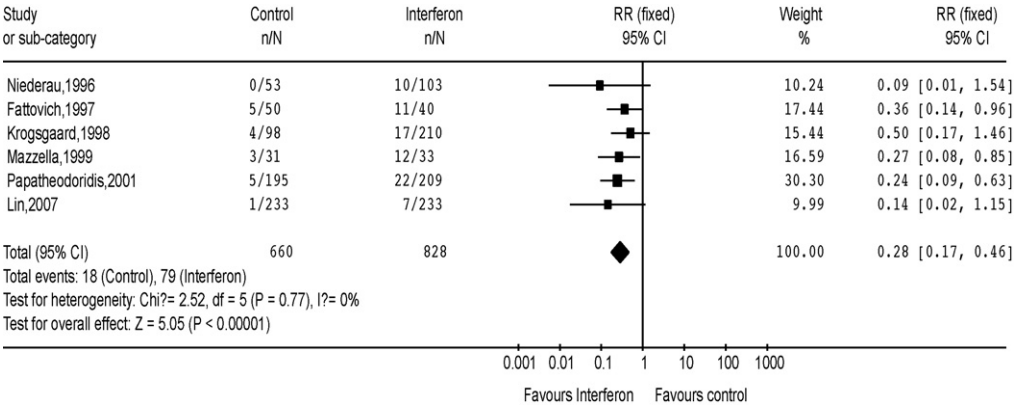

Fig. 2. Meta-analysis of the six trials comparing incidence of HBsAg seroclearance in untreated and interferon treated patients with chronic hepatitis B. Risk Ratio and 95% confidence intervals for each study and the pooled estimate of the treatment effect with its confidence interval are plotted on the graph. Studies are arranged by publication year.

increase the incidence of HBV DNA seroclearance compared with no treatment, while the other one (Lin et al., 2007) showed no significant difference.

3.3. The effect of IFN on HBeAg seroclearance

The effect of IFN on HBeAg seroclearance is shown in Fig. 1. Four studies (Mazzella et al., 1999; Krogsgaard, 1998; Fattovich et al., 1997; Niederau et al., 1996) including 618 patients containing the information about incidence of HBeAg seroclearance were evaluated. The length of follow up ranged from 38.5 months (Niederau et al., 1996) to 86.4 months (Fattovich et al., 1997). Generally, HBeAg seroclearance occurred in 41.5% (160/386) of IFN treated patients and 32.8% (76/232) of untreated patients. IFN treatment significantly increases the incidence of HBeAg seroclearance in two (Mazzella et al., 1999; Niederau et al., 1996) of the four evaluable studies. The heterogeneity test indicates that the variation of trial-specific RRs was statistically significant ($\chi^2 = 11.71$, $P = 0.008$), so the random-effect method was used to combine the results. The combined RR at the end of treatment was 0.66 (95% CI: 0.44, 0.99), and was statistically significant ($P = 0.04$ and <0.05). In addition, when the fixed-effect method was used, the combined RR was 0.65 0.66 (95% CI: 0.53, 0.81), the conclusion was largely unchanged. These results suggest that at the end of follow up for at least three years, IFN treatment significantly increase the incidence of HBeAg seroclearance compared with no treatment.

3.4. Effect of IFN on HBsAg seroclearance

The effect of IFN on HBsAg seroclearance is shown in Fig. 2. Six studies (Papatheodoridis et al., 2001; Mazzella et al., 1999; Krogsgaard, 1998; Fattovich et al., 1997; Niederau et al., 1996; Lin et al., 2007) including 1488 patients containing the information about HBsAg seroclearance were evaluated. The length of follow up ranged from 38.5 months (Niederau et al., 1996) to seven years (Lin et al., 2007). Generally, HBsAg seroclearance occurred in 9.5% (79/828) of IFN treated patients and 2.7% (18/660) of untreated patients. IFN treatment significantly increases the incidence of HBsAg seroclearance in three (Papatheodoridis et al., 2001; Mazzella et al., 1999; Fattovich et al., 1997) of the six evaluable studies. The heterogeneity test indicated that the variation of trial-specific RRs was not statistically significant ($\chi^2 = 2.52$, $P = 0.77$ and >0.10), so the fixed-effect method was used to combine the results. The combined RR at the end of treatment was 0.28 (95% CI: 0.17, 0.46), and was statistically significant ($P < 0.0001$). In addition, the conclusion was largely unchanged when the random-effect method was used.

In these six including studies, the ALT levels were significantly higher in IFN groups than in untreated groups in one study (Papatheodoridis et al., 2001). So we conduct a meta-analysis excluding this study. The heterogeneity test indicates that the variation of trial-specific RRs was not statistically significant ($\chi^2 = 2.27$, $P = 0.69$ and >0.10), so the fixed-effect method was used to combine the results. The combined RR was 0.30 (95% CI: 0.17, 0.53), and was statistically significant ($P < 0.0001$). In addition, the conclusions were largely unchanged when the random-effect method was used.

These results suggest that at the end of follow up for at least three years, IFN treatment significantly increase the incidence of HBsAg seroclearance compared with no treatment.

4. Discussion

Chronic hepatitis due to HBV infection is a serious clinical problem because of its worldwide distribution and potential adverse sequelae. An estimated 350 million persons worldwide are chronically infected with HBV. Chronic hepatitis due to HBV is a progressive disease that may lead to liver cirrhosis and HCC. It is now clear that active HBV replication is the key driver of liver injury and disease progression, thus the aims of treatment of CHB are to achieve sustained suppression of HBV replication and remission of liver disease. The ultimate goal is to prevent cirrhosis, hepatic failure and HCC.

IFN, which has a dual mode of action: antiviral and immunomodulatory, has been used for the treatment of chronic HBV infection for more than two decades. The short-term effect of treatment of chronic hepatitis B (CHB) with IFN is well established. Several controlled clinical trials have shown that approximately 35% of patients with chronic hepatitis B will lose HBV DNA and HBeAg and normalize transaminases, whereas few patients will lose HBsAg (Asselah et al., 2007). A recently published retrospective non-controlled study (Moucari et al., 2009) showed that after a median follow-up of 14 years, 28.9% (28/97) HBeAg positive patients who receive IFN treatment developed HBsAg seroconversion. However, whether IFN treatment can achieve long-term sustain suppression of HBV remains controversial. Thus, we have made a meta-analysis of the available clinical trials to assess long-term effects of IFN on chronic hepatitis B. The results of our study indicate that compared with no treatment, IFN treatment significantly increase the incidence of HBeAg seroclearance and HBsAg seroclearance after long-term follow-up of 3–7 years.

Meta-analysis is traditionally applied and best confined to RCTs. However, there are clinical settings in which an RCT is unfeasible as it would require a large sample size or it would be difficult to perform. In these cases, meta-analytic techniques could be applied to NRCTs (Ozminkowski et al., 1988). NRCTs are subject to many problems that reduce their internal and external validity. Their lack of precision and reliability causes inherent biases towards false positive results. When assessing NRCTs, the most important bias is the likelihood of inappropriate selection of patients, which can lead to incorrect results and spurious associations. In our meta-analysis, most clinical trials enrolled in the study had retrospective and unrandomized design, and individual data from each study (e.g. 'patient-level' data) were not available; thus, it was impossible to perform our own adjustments. Generally, age, male/female ratio, percentage of preexist cirrhosis, and length of follow up were not significant different between IFN groups and untreated groups at baseline in these studies. When analysis HBsAg seroclearance, the ALT levels were significantly higher in IFN groups than in untreated groups in one study (Papatheodoridis et al., 2001). As is known, higher level of ALT was associated with higher incidence of HBV clearance. When excluding this study, the conclusions were largely unchanged. So we presume that although the baseline ALT was different between the IFN group and no treatment group, its influence was insignificant to the conclusion that IFN treatment increases the incidence of HBsAg clearance in patients with CHB.

When comparing effect of IFN treatment vs. no treatment on HBeAg seroclearance, we found a remarkable heterogeneity among the studies. The most prominent heterogeneity was in the difference of magnitude of the treatment effect, so we used random-effect analysis, in which the studies are weighted much more equally. Another potentially important limitation of meta-analysis is publication bias, the fact that not all research is published. Compared to positive studies, negative studies may be less likely to be published and more likely to take longer to be published, which can affect the validity of meta-analysis (Thornton and Lee, 2000). One commonly used method to detect publication bias is the "funnel plot", which is a scatter plot that displays the relationship between the weight of the study (e.g. study size) and the observed effect. In principle, larger studies should display less variability of the treatment effects. Asymmetric appearance, especially due to the absence of smaller negative studies, can suggest unpub-

lished data. However, neither funnel plots nor Egger's test showed evidence for publication bias.

In conclusion, the results of this meta-analysis indicate that IFN increases the incidence of HBeAg seroclearance and HBsAg seroclearance after long-term follow-up of three to seven years.

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